ESTIMATION OF ANTI-DIABETIC TENELIGLIPTIN HYDROBROMIDE HYDRATE BY RP-HPLC AND DERIVATIVE SPECTROSCOPIC METHOD

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ABSTRACT
Simple, accurate, precise and economical HPLC and UV method has been developed and validated for the estimation of teneligliptin hydrobromide hydrate (THH) in bulk and tablet dosage form. Isocratic elution at the flow rate of 1.0 ml/min was employed on a Kromasil 100-5-C8 column at ambient temperature. The mobile phase consisted of Methanol: 0.025M phosphate buffer pH adjusted to 3 with o-phosphoric acid (60:40 v/v). The detection wavelength was at 254nm. Linearity was observed in the concentration range of 10-100 µg/ml. The retention time for Teneligliptin was 4.14 min. In stability testing, teneligliptin was found susceptible to alkali hydrolysis and oxidatative degradation. Because the method could effectively separate the drug from its degradation products, it can be used as a stability indicating method. First order derivative UV spectrophotometric method was also developed using methanol as solvent at analytical λ 261.0 nm. Beer’s law was obeyed in the concentration range of 5-50 µg/ml and \( r^2 =0.9996 \). The proposed methods were validated according to the ICH guidelines. Both the developed methods are accurate and precise and can be used for routine quality control analysis of Teneligliptin in bulk and pharmaceutical formulation. In case of HPLC as well resolved peak is obtained for Teneligliptin after degradation, method is also suitable for stability studies.

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INTRODUCTION

Teneligliptin hydrobromide hydrate i.e. \{(2S,4S)-4-[4-(3-Methyl-1-phenyl-1H-pyrazol-5-yl) piperazin-1-yl] pyrrolidin-2-yl\} (1,3-thiazolidin-3-yl) methanone hemipenta hydrobromide hydrate, is a potent, reversible and selective inhibitor of the enzyme DPP-4 (Dipeptidyl peptidase 4, EC 3.4.14.5) which is involved in the inactivation of the incretin hormones (glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP)). These incretin hormones are rapidly degraded by the enzyme DPP-4. Both incretin hormones are involved in the physiological regulation of glucose homeostasis. GLP-1 and GIP are secreted by the intestine at a low basal level throughout the day and concentrations are increased in response to a meal. GLP-1 and GIP increase insulin biosynthesis and secretion from pancreatic beta cells in the presence of normal and elevated blood glucose levels. Furthermore GLP-1 also reduces glucagon secretion from pancreatic alpha cells, resulting in a reduction in hepatic glucose production. Teneligliptin binds to DPP-4 in a reversible manner and thus leads to an increase and a prolongation of active incretin levels. Teneligliptin glucose dependently increases insulin secretion and lowers glucagon secretion thus resulting in an overall improvement in the glucose homoeostasis. Structure of teneligliptin hydrobromide hydrate is shown in Fig. 1.

![Chemical structure of Teneligliptin hydrobromide hydrate.](http://www.iajpr.com)

Fig. 1: Chemical structure of Teneligliptin hydrobromide hydrate.

Literature survey revealed reported method by UV\(^4\) and by HPLC\(^5\) for analysis of Teneligliptin hydrobromide hydrate and no HPTLC method reported for analysis of Teneligliptin hydrobromide hydrate in bulk and pharmaceutical dosage form. Also analytical method for quantification of teneligliptin in plasma has been reported\(^6\). The purpose of present work is to develop simple, precise, less time consuming, selective and economic high-performance liquid chromatographic method and first order derivative UV spectrophotometric methods for determination of Teneligliptin hydrobromide hydrate in bulk and tablet dosage form. The proposed methods were validated as per ICH guidelines\(^7\).

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC METHOD

MATERIALS AND METHODS

Pure drug, Teneligliptin hydrobromide hydrate was gifted by Lupin limited, Aurangabad. Tablet of teneligliptin Zita plus (20 mg of teneligliptin hydrobromide hydrate, Mfg by:- Glenmark Pharmaceuticals Pvt. Ltd., Solan HP), were purchased from local market. Methanol (HPLC grade) was purchased from Merck Mumbai Ltd., India and double distilled water was used throughout the analysis.

Standard stock solution

Accurately weighed quantity of Teneligliptin hydrobromide hydrate 10 mg was transferred to 100.0 ml volumetric flask, dissolved and diluted to the mark with methanol (Concentration 100 µg/ml of THH).

Selection of mobile phase

Aliquot portion of standard stock solution was appropriately diluted with mobile phase to obtain final concentration of 20µg/ml. The diluted standard solution was filtered through 0.2 µ membrane filter. The filtrate was injected into the HPLC system and run in different solvent systems. Mixture of different solvents with varying polarity were tried in order to determine optimum chromatographic conditions for obtaining a sharp peak of THH with minimal tailing. After several permutation and combination, it was found that mixture of methanol and 0.025M buffer pH 3.0 gives satisfactory results as compared to other mobile phases. Finally, the optimal composition of the mobile phase Methanol: 0.025M Phosphate buffer pH adjusted to 3 with o-phosphoric acid (60:40 v/v), and flow rate 1 ml/min was selected as it gave sharp symmetric peak for THH with minimal tailing and with desired elution graph. Retention time of THH was found to be 4.14 minutes.
Selection of analytical wavelength

Aliquot portion of standard stock solution was appropriately diluted with mobile phase to obtain final concentration of 20µg/ml of THH. The solution was scanned using double beam UV-Visible Spectrophotometer-1700 in the spectrum mode between the wavelength ranges of 400 nm to 200 nm against mobile phase as blank. The wavelength selected was 254 nm as THH showed significant absorbance at this wavelength. Typical chromatogram obtained is shown in Fig. 2.

![Typical chromatogram of Teneligliptin](image)

**Fig. 2: Typical chromatogram of Teneligliptin.**

**Instrumentation and optimized chromatographic conditions**

Instrument used is Agilent 1120 compact LC binary gradient system. The software used is EZ-chrome elite.

- **HPLC Column**: Kromasil 100-5-C8, (250 mm X 4.6 mm, 5µm)
- **Column temperature**: Ambient temperature
- **Mobile Phase**: Methanol: 0.025M Phosphate buffer pH 3 (60:40 v/v)
- **Flow rate**: 1.0 ml/min
- **UV detection**: 254 nm
- **Injection volume**: 20 µl
- **Run time**: 10 min

**Calibration plot for Teneligliptin hydrobromide hydrate**

Ten linearity test solutions for THH were prepared as follows:

Accurately weighed quantity (10 mg) of THH, was transferred to 100.0 ml volumetric flask, added 30 ml of mobile phase and ultrasonicated for 10 minutes, volume was then made up to the mark with mobile phase. Aliquot portions of above solution 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0ml were diluted individually to 10.0 ml with mobile phase (Concentration 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µg/ml, respectively).

Each solution was then filtered through 0.2 µ membrane filter.

The diluted solutions were injected (20 µl) into the HPLC system and chromatographed using optimum chromatographic conditions. The peak area of THH was measured at 254 nm. Each solution was injected and chromatographed in triplicate. Mean peak areas were calculated for each drug concentration.

**Preparation of sample solution for analysis of marketed tablet formulation**

Twenty tablets were weighed, average weight was calculated and crushed to obtain fine powder. Accurately weighed quantity of tablet powder equivalent to about 10mg THH was transferred to 50.0 ml volumetric flask, added 20ml of mobile phase and ultrasonicated for 20 min, volume was then made up to the mark with mobile phase. The solution was then mixed and filter through Whatmann filter paper no. 42. From the filtrate, 5.0 ml solution was diluted to 25.0 ml with mobile phase. The solution was filtered through 0.2 µ membrane filter.

Equal volume (20 µL) of standard and sample solution was injected into the HPLC system and chromatographed using optimum chromatographic conditions. Each solution was injected and chromatographed in triplicate. The chromatograms were recorded and peak area of THH was measured at 254 nm. Amount for THH (in mg/tablet) was estimated by comparing mean peak area of sample with that of the standard and percent label claim was calculated.

**Method validation**

The method was validated in compliance with ICH guidelines.
Accuracy
An accurately weighed quantity of tablet powder equivalent to about 10 mg THH was transferred individually in nine different 50.0 ml volumetric flasks. Add 8 mg, 10mg and 12 mg of THH pure drug to the sample into three volumetric flasks respectively for 80 %, 100 % and 120 % level of recovery. All dilutions were performed as described in preparation of sample solution of marked formulation with mobile phase. Solutions were prepared in triplicate and analysed. Accuracy was determined and expressed as percent recovery.

Precision
To ascertain repeatability and reproducibility of the method precision studies were performed. Sample solution was prepared and analysed in the similar manner as described under analysis of the marketed formulation. Intra-day precision was determined by analyzing a sample solution at three different time intervals on the same day and inter-day precision was determined by analyzing a sample solution on three consecutive days.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)
The LOD and LOQ were separately determined based on the standard deviation of response of the calibration curve. The standard deviation of y-intercept and mean of slope of the calibration curves were used to calculate the LOD and LOQ.

Robustness
To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of changes in mobile phase composition and flow rate on retention time and tailing factor of drug peak was studied. The mobile phase composition was changed in ± 2ml proportion and the flow rate was varied by ± 0.1 ml/min, of optimized chromatographic condition. The solution containing THH was injected into the HPLC system and chromatographed under varied conditions.

Solution Stability study
Stability of sample solution was determined by injecting (in triplicate) tablet sample solution at different time intervals. i.e., at 0 minutes, 1 hr, 3 hr, 6 hr, 8 hr and 24 hr and chromatographed under optimum conditions. Sample solution was injected and chromatographed in duplicate at each time interval. The retention time and peak area of THH was recorded.

Forced degradation studies
Accurately weighed quantity of tablet powder equivalent to about 10 mg of THH was transferred separately to six different 50.0 ml volumetric flask, (flask no. 1, 2, 3, 4, 5 and 6). To flask no. 1, 2 and 3, added 3.0 ml of methanol as cosolvent was added followed by addition of 5.0 ml 5 M HCl, 0.05 M NaOH and 3 % H₂O₂ to flask no.1, 2, and 3, respectively. For neutral hydrolysis, 5.0 ml of water was added to flask no.4. The content of flask no. 1, 2, 3 and 4 were heated on water bath at 80°C for 3 hrs. Flask no. 5 containing tablet powder was kept in hot air oven at 60°C for 24hrs to study the effect of heat on tablet sample (heat degradation). The forced degradation studies were performed in dark to exclude the possible degradative effect of light. Flask no. 6 containing tablet power was exposed to UV-radiations for 24 hrs to study the effect of light on tablet sample (photo degradation). After stipulated time interval, all the flask were removed and cooled to room temperature. The samples were then treated and analysed in similar manner as discussed under analysis of marketed formulation.

RESULT AND DISCUSSION
Linearity
Peak areas were found to have good linear relationship with the concentration. Teneligliptin hydrobromide hydrate was found to give linear detector response in the concentration range of 10-100 µg/ml (shown in Fig.3). The straight line equation and coefficient of correlation for THH calibration curve was $y = 310294x + 17925$ and $r^2 = 0.9995$ respectively.

![Fig. 3: Calibration curve of Teneligliptin at 254 nm.](www.iajpr.com)
Analysis of marketed formulation

Analysis of marketed formulation containing THH (20 mg) was carried out and results are expressed as percentage amount of the label claim. There was no interference from the excipients. The THH content was found to be close to 100% and the result is summarized in Table 1. The low SD value indicated the suitability of this method for routine analysis.

<table>
<thead>
<tr>
<th>Brand Name</th>
<th>Label Claim (mg)</th>
<th>Amount Estimated* (mg/tab)</th>
<th>% Label Claim* ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zita plus</td>
<td>20</td>
<td>20.155</td>
<td>100.778 ± 1.078</td>
</tr>
</tbody>
</table>

*mean of six observations.

Accuracy

To ascertain the accuracy of proposed method, recovery studies were carried out by standard addition method and the results are expressed as percent recovery. The mean percentage recovery for each compound was calculated at each concentration level and reported with its standard deviation. The percentage recovery at three levels (80%, 100% and 120%) was found to be satisfactory (Table 2) indicating the accuracy of developed method.

<table>
<thead>
<tr>
<th>% Level of Recovery ± SD</th>
<th>Percent recovery* ± SD</th>
<th>% RSD</th>
<th>Mean % Recovery** ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>100.103 ± 1.093</td>
<td>1.092</td>
<td>100.453 ± 1.089</td>
</tr>
<tr>
<td>100</td>
<td>100.23 ± 0.745</td>
<td>0.743</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>101.026 ± 0.95</td>
<td>0.94</td>
<td></td>
</tr>
</tbody>
</table>

*mean of three observations.

**mean of three observations.

Precision

Precision was evaluated by carrying out independent sample preparation of a single lot of formulation on same day 3 times and on three different days. Standard deviation and percentage relative standard deviation (% RSD) was found to be less than 2% for intraday and interday precision (Table 3) indicating the repeatability and reproducibility of the developed method.

<table>
<thead>
<tr>
<th>Precision</th>
<th>% label claim* ± SD</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-day precision</td>
<td>99.983 ± 0.870</td>
<td>0.87</td>
</tr>
<tr>
<td>Inter-day precision</td>
<td>101.5 ± 0.915</td>
<td>0.901</td>
</tr>
</tbody>
</table>

*mean of three observations.

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ values for THH was found to be 3.46 and 10.48 µg/ml, respectively. The low LOD and LOQ values for THH indicate the sensitivity of the method.

Robustness

The effect of change in mobile phase composition (± 2 ml) and flow rate (± 0.1 ml/min) on the tailing factor and retention time of drug was studied. The method was found to be unaffected by small changes in method parameters with % RSD for tailing factor and retention time under varied method parameters was less than 2.0%. The developed method is considered to be robust.

Solution stability study

The retention time and peak area of THH was recorded at different time intervals. The %RSD of peak area was less than 2.0% indicating that the standard solution was stable throughout the study time.

Forced degradation studies

Intentional degradation of THH was tried under different stress conditions such as acid hydrolysis, alkaline hydrolysis, oxidation, neutral hydrolysis, heat and exposure to UV radiations. Teneligliptin was found to degrade more in alkaline and oxidative stress conditions as compared to acidic conditions. The percent assay of active substance and the retention time (Rt) values of degradation products are given in Table 4. Chromatogram of acid, alkaline and hydrogen peroxide treated samples are shown in Fig.4, 5 and 6 respectively.
Table 4: Results of forced degradation study by RP-HPLC.

<table>
<thead>
<tr>
<th>Stress condition</th>
<th>Percent assay of active substance</th>
<th>Rt of degraded product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid (5.0 M HCl, 80ºC for 3 hrs)</td>
<td>90.546</td>
<td>2.537, 2.643, 3.443, 3.703, 3.937</td>
</tr>
<tr>
<td>Alkali (0.05 M NaOH, 80ºC for 3 hrs)</td>
<td>65.252</td>
<td>3.676</td>
</tr>
<tr>
<td>Oxidation (3 % H₂O₂, 80ºC for 3 hrs)</td>
<td>79.99</td>
<td>2.697, 2.973, 3.307, 3.787</td>
</tr>
<tr>
<td>Neutral (Distilled water, 80ºC for 3 hrs)</td>
<td>99.157</td>
<td>-</td>
</tr>
<tr>
<td>Heat (60ºC for 24 hrs)</td>
<td>100.081</td>
<td>-</td>
</tr>
<tr>
<td>UV-Exposure (254nm for 24 hrs)</td>
<td>99.885</td>
<td>-</td>
</tr>
</tbody>
</table>

![Fig. 4: Chromatogram of acid (5.0 M HCl) treated sample.](image)

![Fig. 5: Chromatogram of alkali (0.05 M NaOH) treated sample.](image)

![Fig. 6: Chromatogram of peroxide (3% H₂O₂) treated sample.](image)
FIRST ORDER DERIVATIVE UV SPECTROPHOTOMETRIC METHOD

Determination of analytical wavelength

The standard stock solution of 100 μg/ml of teneligliptin hydrobromide hydrate was prepared by weighing 10mg of the drug, taken in 100 ml volumetric flask and diluted with methanol. Take 3 ml of above solution and dilute it to 10 ml with methanol. The resulting solution was scanned in first order derivative mode in the range of 200-400 nm to determine the analytical wavelength. The wavelength selected for analysis was 261.0 nm. The first order derivative spectra of THH is shown in Fig. 7.

Method validation

The proposed method was validated for different parameters like linearity, precision, accuracy, ruggedness, robustness, LOD, LOQ and assay.

Linearity Study

The linearity was determined by plotting concentration against corresponding absorbance. Standard stock solution, 100μg/ml were further diluted with methanol to obtain 5, 10, 20, 30, 40, 50μg/ml solutions. The absorbance of resulting solutions were taken in first order derivative mode at 261.0 nm. The calibration curve was constructed by plotting absorbance versus concentration and the coefficient of correlation and straight line equation were calculated.

Intra-day precision study

Aliquot (7.5ml) of the 100μg/ml THH stock solution was taken in 25 ml volumetric flask and diluted with methanol to obtain 30 μg/ml. Triplicate absorbance measurements were made for three times on same day in first order derivative mode at 261.0 nm and the percentage RSD was calculated.

Inter-day precision study

The selected concentration for the intra-day precision study was again analysed for three consecutive days and the percentage RSD was calculated.

Accuracy

Accuracy of the method was calculated by recovery studies at three different levels (80%, 100% and 120%) in triplicate at each level by standard addition method to study the accuracy of the method and to check the interference from excipients. The percentage recovery in each case was calculated.

Ruggedness

The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same homogeneous samples under a variety of conditions such as different laboratories, different analysts, different instruments, different lots of reagents, different elapsed assay times, different assay temperatures, different days, etc. Ruggedness is normally expressed as the lack of influence of operational and environmental factors of the analytical method. It was determined by carrying out analysis on different instruments and different analyst. The absorbance and assay was carried out three times.

Robustness

The robustness of an analytical procedure is the measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It was determined by carrying out the analysis at λmax ± 1 nm. The absorbance and assay was carried out three times.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were separately determined based on the standard deviation of response of the calibration curve. The standard deviation of y-intercept and mean of slope of the calibration curves were used to calculate the LOD and LOQ.
Assay of Marketed Formulation

Accurately weighed quantity of tablet powder equivalent to about 10.0 mg of THH was transferred to 100.0 ml volumetric flask, added 30 ml methanol and ultrasonicated for 10 min, volume was then made up to the mark with methanol. The solution was mixed and filtered through Whatmann filter paper no. 42. Then 7.5 ml filtrate was diluted to 25.0 ml with methanol. Absorbance of resulting solution was measured in first order derivative mode at 261.0 nm. The concentration of THH in the sample was calculated by using straight line equation of calibration curve. The % assay of the drug was calculated.

RESULT AND DISCUSSION

Linearity

Teneligliptin hydrobromide hydrate was found to give linear detector response in the concentration range of 5-50 µg/ml (shown in Fig. 8). The straight line equation and coefficient of correlation for THH calibration curve was $y = -0.0007x - 0.0015$ and $r^2 = 0.9996$ respectively.

![Fig. 8: Calibration curve of THH at 261.0 nm.](chart)

The results of the validation parameters of the proposed first order derivative UV method are summarized in Table 5, 6, 7, 8, 9 and 10.

Table 5: Results of Accuracy by UV method.

<table>
<thead>
<tr>
<th>% Level of Recovery</th>
<th>Percent recovery* ± SD</th>
<th>%RSD</th>
<th>Mean % Recovery** ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>101.39 ± 1.30</td>
<td>1.28</td>
<td>100.02 ± 1.26</td>
</tr>
<tr>
<td>100</td>
<td>98.66 ± 0.942</td>
<td>0.955</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>101.79 ± 1.20</td>
<td>1.18</td>
<td></td>
</tr>
</tbody>
</table>
*mean of three observations
**mean of nine observations

Table 6: Results of precision studies by UV method.

<table>
<thead>
<tr>
<th>Precision</th>
<th>% Label claim* ± SD</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-day precision</td>
<td>99.58 ± 0.193</td>
<td>0.194</td>
</tr>
<tr>
<td>Inter-day precision</td>
<td>99.82 ± 0.110</td>
<td>0.11</td>
</tr>
</tbody>
</table>
*mean of three observations

Table 7: Results of LOD and LOQ by UV method.

<table>
<thead>
<tr>
<th>Limit of Detection (µg/ml)</th>
<th>3.76</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limit of Quantification (µg/ml)</td>
<td>11.4</td>
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</table>

Table 8: Results of robustness studies by UV method.

<table>
<thead>
<tr>
<th>Robustness</th>
<th>Label claim* ± SD</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>At 260 nm</td>
<td>99.52 ± 0.11</td>
<td>0.11</td>
</tr>
<tr>
<td>At 262 nm</td>
<td>99.11 ± 0.25</td>
<td>0.26</td>
</tr>
</tbody>
</table>
*mean of three observations.
Table 9: Results of ruggedness studies by UV method.

<table>
<thead>
<tr>
<th>Ruggedness</th>
<th>Instrument</th>
<th>Analyst</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>% Label Claim</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>% Label Claim</td>
<td>99.759</td>
<td>100.317</td>
<td>100.033</td>
<td>99.824</td>
<td></td>
</tr>
<tr>
<td>S.D. (±)</td>
<td>0.168</td>
<td>0.266</td>
<td>0.342</td>
<td>0.159</td>
<td></td>
</tr>
</tbody>
</table>

*mean of three observations.

Table 10: Results of analysis of marketed formulation by UV method.

<table>
<thead>
<tr>
<th>Brand Name</th>
<th>Label Claim (mg)</th>
<th>Amount Estimated* (mg/tab)</th>
<th>%Label Claim*± SD</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Zita plus</td>
<td>20</td>
<td>19.989</td>
<td>99.949 ± 0.221</td>
<td></td>
</tr>
</tbody>
</table>

*mean of six observations.

CONCLUSION

Based on the results obtained it is concluded that both the methods are sensitive, accurate, precise and reproducible, where teneligliptin hydrobromide hydrate can be determined in bulk and in pharmaceutical formulation without interference from the excipients. The proposed HPLC method gave sharp peak for THH and complies system suitability parameters. The HPLC method was also able to selectively quantitate THH in presence of the degradation products obtained in forced degradation study. Hence, the methods can be employed as a stability indicating one. The first order derivative UV method proposed is also a good alternative for analysis of THH. ICH guidelines were followed throughout method validation and the suggested method can be applied for routine quality control analysis of pharmaceutical formulation containing the drug.

Recommended future research: The HPLC method can be further extended to characterize the structure of the degradation products.

LIST OF ABBREVIATIONS

UV – Ultra violet
HPTLC – High performance thin layer chromatography
HPLC – High performance liquid chromatography
GLP-1 – Glucagon-like peptide-1
GIP – Glucose-dependent insulinoetric polypeptide
DPP4 – Dipeptidyl peptidase 4
THH – Teneligliptin hydrobromide hydrate
SD – Standard deviation
RSD – Relative standard deviation
ICH – International conference on harmonization

CONFLICT OF INTREST

None

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